



71914.ST25.txt
SEQUENCE LISTING

<110> COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
Rao, Nalam M
Acharya, Priyamvada

<120> STABLE GENE VARIANTS OF LIPASES

<130> 71914

<140> US 10/768,951

<141> 2004-01-29

<160> 34

<170> PatentIn version 3.3

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<212> PRT

<213> Bacillus subtilis

<220>

<221> AMINO ACIDS

<222> (1)..(181)

<223> enzyme sequence

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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
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Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu

145 150 160

Tyr Ser Ser Gln Val Asn Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
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Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
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<222> (1)..(181)
<223> Protein sequence

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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
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Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Val Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Thr Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
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Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
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Gly Gln Asn Thr Asn
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 <223> Protein Sequence

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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
 20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
 35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
 50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
 65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
 85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
 100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
 115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
 130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
 145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
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Gly Gln Asn Thr Asn
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Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
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Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
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Gly Gln Asn Thr Asn
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<213> Bacillus subtilis

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<222> (1)..(181)
<223> Protein sequence

<400> 7

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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
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Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Ser Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
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<213> Bacillus subtilis

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Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

71914.ST25.txt

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
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Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
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Gly Gln Asn Thr Asn
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20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Ala Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
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Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

71914.ST25.txt

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asn Gln Lys Ile Leu Tyr Thr Ser
115 120 125

Ile Tyr Ser Ser Ala Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Val Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
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<223> Protein sequence

<400> 11

Met Ala Glu His Asn Pro Val Val Met Val His Gly Ile Gly Gly Ala
1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Val Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Leu Pro Gly Thr Asp Pro Asp Gln Lys Ile Leu Tyr Thr Ser
115 120 125

71914.ST25.txt

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
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Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
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Gly Gln Asn Thr Asn
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<400> 12

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1 5 10 15

Ser Phe Asn Phe Ala Gly Ile Lys Ser Tyr Leu Val Ser Gln Gly Trp
20 25 30

Ser Arg Asp Lys Leu Tyr Ala Val Asp Phe Trp Asp Lys Thr Gly Thr
35 40 45

Asn Tyr Asn Asn Gly Pro Val Leu Ser Arg Phe Val Gln Lys Val Leu
50 55 60

Asp Glu Thr Gly Ala Lys Lys Val Asp Ile Val Ala His Ser Met Gly
65 70 75 80

Gly Ala Asn Thr Leu Tyr Tyr Ile Lys Asn Leu Asp Gly Gly Asn Lys
85 90 95

Val Ala Asn Val Val Thr Leu Gly Gly Ala Asn Arg Leu Thr Thr Gly
100 105 110

Lys Ala Pro Pro Gly Thr Asp Pro Asp Gln Lys Ile Leu Tyr Thr Ser
115 120 125

71914.ST25.txt

Ile Tyr Ser Ser Asp Asp Met Ile Val Met Asn Tyr Leu Ser Arg Leu
130 135 140

Asp Gly Ala Arg Asn Val Gln Ile His Gly Gly His Ile Gly Leu Leu
145 150 155 160

Tyr Ser Ser Gln Val Tyr Ser Leu Ile Lys Glu Gly Leu Asn Gly Gly
165 170 175

Gly Gln Asn Thr Asn
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<400> 14
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<210> 15
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<223> Primer

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<210> 16
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<212> DNA
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<400> 17
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23

<210> 18
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<223> Primer

<400> 21
tgacacagga aacagctatg ac

22

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<213> Artificial Sequence

<220>
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<400> 22

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24

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 aatcaaaaga ttttatacac atccatttac agcagtgccg atatgattgt catgaattac 420
 ttatcaagat tagatggtgc tagaaacggt caaatccatg gcgttggaca catcggcctt 480
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 gatttttggg acaagacagg cacaaattat aacaatggac cggtattatc acgatttggtg 180
 caaaagggtt tagatgaaac ggggtgcgaaa aaagtggata ttgtcgctca cagcatgggg 240
 ggcgcgaaca cactttacta cataaaaaat ctggacggcg gaaataaagt tgcaaacgctc 300
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 aatcaaaaga ttttatacac atccatttac agcagtgccg atatgattgt catgaattac 420
 ttatcaagat tagatggtgc tagaaacggt caaatccatg gcgttggaca catcggcctt 480
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 acgaattaat ga 552

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 <223> lipase gene variant

71914.ST25.txt

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gatttttggg acaagacagg cacaaattat aacaatggac cggattatc acgatttgtg    180
caaaagggtt tagatgaaac ggggtgtgaa aaagtggata ttgtcgctca cagcatgggg    240
ggcggaaca cactttacta cataaaaaat ctggacggcg gaaataaagt tgcaaacgct    300
gtgacggttg gcggcgcgaa ccgtttgacg acaggcaagg cgcttcggg aacagatcca    360
aatcaaaaga ttttatacac atccatttac agcagtgcg atatgattgt catgaattac    420
ttatcaagat tagatgggtc tagaaacggt caaatccatg gcgttggaac catcggcctt    480
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gatttttggg acaagacagg cacaaattat aacaatggac cggattatc acgatttgtg    180
caaaagggtt tagatgaaac ggggtacgaa aaagtggata ttgtcgctca cagcatgggg    240
ggcggaaca cactttacta cataaaaaat ctggacggcg gaaataaagt tgcaaacgct    300
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ttatcaagat tagatgggtc tagaaacggt caaatccatg gcgttggaac catcggcctt    480
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gatttttggg acaagacagg cacaaattat aacaatggac cggattatc acgatttgtg    180

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caaaagggttt tagatgaaac ggggtgcgaaa aaagtggata ttgtcgctca cagcatgggg	240
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gtgacggttg gcggcgcgaa ccgtttgacg acaggcaagg cgcttccggg aacagatcca	360
aatcaaaaga ttttatacac atccatttac agcagtgcg atatgattgt catgaattac	420
ttatcaagat tagatggtgc tagaaacgtt caaatccatg gcgttggaca catcggcctt	480
ctgtacagca gccaaagtcta cagcctgatt aaagaaggcg tgaacggcg gggccagaat	540
acgaattaat ga	552

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gattttttggg acaagacagg cacaaattat aacaatggac cggtattatc acgatttggtg	180
caaaagggttt tagatgaaac ggggtgcgaaa aaagtggata ttgtcgctca cagcatgggg	240
ggcgcgaaca cactttacta cataaaaaat ctggacggcg gaaataaagt tgcaaacgtc	300
gtgacggttg gcggcgcgaa ccgtttgacg acaggcaagg cgccctccgg gaacagatcc	360
aatcaaaag attttataca catccattta cagcagtgc gatatgattg tcatgaatta	420
cttatcaaga ttagatggtg ctagaaacgt tcaaatccat ggcgttggac acatcggcct	480
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gattttttggg acaagacagg cacaaattat aacaatggac cggtattatc acgatttggtg	180
caaaagggttt tagatgaaac ggggtgcgaaa aaagtggata ttgtcgctca cagcatgggg	240
ggcgcgaaca cactttacta cataaaaaat ctggacggcg gaaataaagt tgcaaacgtc	300
gtgacggttg gcggcgcgaa ccgtttgacg acaggcaagg cgcttccggg aacagatcca	360

71914.ST25.txt

aatcaaaaga ttttatacac atccatttac agcagtgccg atatgattgt catgaattac 420
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<220>
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<220>
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 ttatcaagat tagatggtgc tagaaacggt caaatccatg gcgttggaca catcggcctt 480
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 ttatcaagat tagttggtgc tagaaacggt caaatccatg gcgttggaca catcggcctt 480
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 acgaattaat ga 552

<210> 33
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<223> lipase gene variant

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tacgaattaa tga	553

EXHIBIT B

**Applicants: Nalam Madhusdhana Rao and
Piyamvada Acharya**

U.S. Serial No.: 10/768,951

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